The Acute Salivary Ghrelin Response to a Psychosocial Stress Is Enhanced in Symptomatic Patients with Bulimia Nervosa: A Pilot Study

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Key Words

Bulimia nervosa • Binge eating • Cortisol • Ghrelin • Stress

Abstract

Background: Stress is a precipitating factor for both binge eating and bulimia nervosa (BN); however, the biological mechanisms through which it may trigger binge eating are poorly understood. There is evidence that the adrenal hormone cortisol and the gastric peptide ghrelin might be involved in stress-induced food ingestion. We hypothesized that symptomatic patients with BN might disclose deranged responses of ghrelin and/or cortisol to stressors and that this could be related to their binge-eating behaviour. **Methods:** Here we investigated salivary cortisol and ghrelin responses to the Trier Social Stress Test (TSST) in 10 women with acute BN and 10 age-matched healthy females. Eating-related psychopathology and behaviours were assessed by self-report measures. **Results:** No significant differences emerged between bulimic patients and healthy controls in the pre-stress salivary levels of both cortisol and ghrelin. The BN patients displayed normal cortisol but enhanced ghrelin responses to TSST. No significant correlations emerged between stress-induced salivary hormone changes and self-report measures of binge eating. **Conclusion:** To our knowledge, this is

the first study showing deranged salivary ghrelin reactivity to a psychosocial stressor in symptomatic patients with BN. The extent to which this could contribute to the binge-eating behaviour of BN subjects awaits clarification.

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Introduction

Bulimia nervosa (BN) is an eating disorder characterized by episodes of massive food ingestion with associated feelings of loss of control (binge eating), followed by vomiting, prolonged starvation and other compensatory behaviours aiming to reduce energy intake or to increase its expenditure, generally with no pathological change in body weight.

Stress is a documented precipitating factor for both binge eating and BN [1–4]. The affect regulation theory of binge eating posits that many individuals binge in response to stress or negative mood states in an attempt to reduce anxiety and/or negative emotions by increasing feelings of pleasure derived by the ingestion of food or by distracting oneself from one's problems [5–7]. Alternatively, the restraint theory conceptualizes that individuals who restrict food intake to control their body weight

are particularly susceptible to binge eating, especially when exposed to stressful events, which may favour the disinhibition of their restrained eating [8]. Therefore, although evidence has been provided that stress is also associated with decreased food intake in humans [9], it seems to play a crucial role in binge eating. Given this background, understanding the biological mechanisms through which stress may trigger binge eating may help to prevent and/or treat this abnormal behaviour.

The adrenal hormone cortisol and the gastric peptide ghrelin might be involved in stress-induced food ingestion. Indeed, cortisol is the main endogenous mediator of the body's response to stressors [10] and has been shown to promote food intake in both animals and humans [11, 12]. Ghrelin is an orexigenic peptide, which not only stimulates the ingestion of food in a condition of energy deprivation [13] but also mediates the rewarding aspect of food intake [14]. Moreover, acute stress has been reported to increase the production of gastric ghrelin in rodents [15, 16]. Finally, dysregulations in both ghrelin and hypothalamus-pituitary-adrenal (HPA) axis physiology have been largely documented in the acute phase of BN [17–19].

We hypothesized that patients with BN might disclose deranged responses of ghrelin and/or cortisol to stressors and that this could be related to their binge-eating behaviour. Therefore, we measured salivary cortisol and ghrelin responses to a psychosocial stress, represented by the Trier Social Stress Test (TSST) [20], in symptomatic patients with BN compared to age- and sex-matched healthy controls.

Patients and Methods

Twenty women were recruited for the study. They included 10 outpatients attending the Eating Disorder Center of the Department of Psychiatry of the University of Naples SUN and 10 healthy controls. According to the DSM-IV criteria, the patients fulfilled the diagnosis of BN purging subtype. Diagnostic assessment was made by a trained interviewer using the Structured Clinical Interview for Axis I DSM-IV disorders – patient edition [21]. At the time of the study, the patients had never taken psychotropic medications and had no comorbid Axis I disorders.

The control women were drug free and mentally healthy, as assessed by the Structured Clinical Interview for Axis I DSM-IV disorders – non-patient edition [22]; moreover, information on their eating habits was carefully collected by an ad hoc structured clinical interview to exclude eating disorders, which could have occurred at subclinical severity and thereby confound the results. In both the patients and the controls, eating-related psychopathology and behaviours were assessed by means of the Bulimia Investigation Test of Edinburgh (BITE) [23] and the Three Factor Eating Questionnaire (TFEQ) [24].

Procedure

The experimental protocol was approved by the local ethics committee and all subjects gave their written consent after being fully informed of the nature and procedure of the study. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Both the BN patients and the healthy women were tested in the follicular phase of their menstrual cycle (day 5–10 from menses).

The TSSTs were performed between 3:30 and 5:00 pm, since the HPA axis activity is relatively stable at this time of the day, and changes induced by experimental challenges are therefore more evident. The subjects arrived to our experiment unit at 3:30 pm and spent about 20 min resting in experimental room No. 1. At the end of the resting period, they were instructed about the procedure of the TSST; they were told that they first had to deliver a speech for a job application to a committee, for which they would have 5 min to prepare and 5 min to deliver, and that their performance was recorded on a video cassette recorder; after finishing the speech they had to perform an arithmetic task within 5 min. The subjects were then led to experimental room No. 2, where behind a table were seated a group of three people who were referred to as 'the committee'. The committee was dressed in white coats and was introduced as consisting of psychiatrists and psychologists especially trained to monitor and analyse verbal and non-verbal behaviour. If the subject finished her speech in less than 5 min, standardized questions were used. Immediately after finishing the speech, the participants were asked to serially subtract the number 13 from 2,083 as quickly and as accurately as possible within 5 min. On every failure, one member of the committee interfered and the participants had to start again at 2,083. Saliva samples were collected at the end of the resting period ($T = 0$), at the end of the TSST ($T = 10$) and 15 ($T = 25$), 30 ($T = 40$) and 50 min ($T = 60$) after finishing the test.

The participants were instructed to remove lipstick and to refrain from eating, drinking (except water), smoking and brushing teeth at least 60 min before sampling as well as during the testing session. Saliva samples were collected into Salivette tubes, centrifuged for 15 min at 4,000 g at 4°C immediately after collection and then stored in aliquots at –80°C until assayed for cortisol and ghrelin levels.

Biochemical Analyses

Saliva cortisol concentrations were determined by the enzyme immunoassay method, using a commercially available ELISA kit (Biochem Immunosystem, Milan, Italy). Intra- and inter-assay coefficients of variation were <8 and <8.7%, respectively. Saliva ghrelin concentrations were measured by a commercial ELISA kit (Phoenix Pharmaceuticals Inc., Burlingame, Calif., USA); intra- and inter-assay coefficients of variation were <5 and <9%, respectively.

Data Analysis

The BMDP statistical software package [25] was used for data analysis. A two-way analysis of variance (ANOVA) with repeated measures was employed to test differences in saliva cortisol and ghrelin responses to the TSST between BN patients and healthy controls. Between-group differences at each time point were evaluated by Tukey's post hoc test. One-way ANOVA was adopted to

Table 1. Clinical and demographic characteristics and pre-stress salivary hormone levels of the study sample

	Healthy women	Women with BN	$F_{1, 18}$	p
Age, years	24.1 $\pm$ 3.9	25.3 $\pm$ 4.2	0.42	0.5
BMI	22.2 $\pm$ 2.4	21.7 $\pm$ 3.6	0.16	0.6
Past minimum BMI	20.4 $\pm$ 3.8	18.3 $\pm$ 3.1	2.52	0.1
Past maximum BMI	24.2 $\pm$ 3.5	25.0 $\pm$ 5.0	0.19	0.6
Duration of illness, years	–	7.4 $\pm$ 4.0		
Weekly frequency of bingeing	–	8.8 $\pm$ 9.2 (2–28)		
BITE symptom score	4.3 $\pm$ 2.3	22.1 $\pm$ 6.0	76.45	<0.00001
BITE gravity score	0.8 $\pm$ 1.2	11.5 $\pm$ 4.5	52.56	<0.00001
BITE total score	5.1 $\pm$ 3.1	33.6 $\pm$ 9.8	88.58	<0.00001
TFEQ factor 1 score	7.1 $\pm$ 4.5	16.2 $\pm$ 3.0	27.97	<0.0001
TFEQ factor 2 score	4.8 $\pm$ 2.2	10.7 $\pm$ 2.7	28.05	<0.0001
TFEQ factor 3 score	4.2 $\pm$ 2.2	8.0 $\pm$ 4.2	5.86	0.02
Pre-stress salivary cortisol, nmol/l	7.7 $\pm$ 2.2	6.4 $\pm$ 3.1	1.25	0.2
Pre-stress salivary ghrelin, ng/ml	0.12 $\pm$ 0.10	0.11 $\pm$ 0.07	0.06	0.8

Values in parentheses indicate range.

Fig. 1. Salivary cortisol response to the TSST in drug-free patients with BN and healthy controls. Data are expressed as mean $\pm$ SEM of saliva absolute values.

assess differences in clinical, demographic and anthropometric variables and pre-stress hormone levels between the two groups. Correlations between TSST-induced responses in saliva hormone levels and the subjects' clinical characteristics were assessed by Pearson's correlation test.

Results

No significant difference was found in anthropometric and demographic parameters between the BN patients and the healthy women (table 1). Instead, as expected, the BN patients scored significantly higher than the healthy women on BITE symptom and gravity subitems and BITE total scores as well as on TFEQ factor 1, which measures the restraint behaviour, TFEQ factor 2, which measures the tendency to lose control over eating, and TFEQ factor 3, which measures the hunger sensation (table 1).

Pre-stress levels of both salivary cortisol and ghrelin did not differ significantly between the BN patients and the healthy controls (table 1).

Saliva Cortisol Response to TSST

Two-way ANOVA with repeated measures disclosed no significant effect for group ($F_{1, 18} = 1.89$, $p = 0.1$) and no significant group $\times$ time interaction ($F_{4, 72} = 0.16$, $p = 0.9$) but a significant effect for time ($F_{4, 72} = 9.18$, $p < 0.00001$), indicating that saliva cortisol levels significantly increased after the TSST with no significant differences between the BN and the control women (fig. 1).

Saliva Ghrelin Response to TSST

Two-way ANOVA with repeated measures disclosed a trend toward a significant effect for group ($F_{1, 18} = 3.97$, $p = 0.06$), a significant effect for time ($F_{4, 72} = 8.25$, $p < 0.00001$) and a significant group $\times$ time interaction

Fig. 2. Salivary ghrelin response to the TSST in drug-free patients with BN and healthy controls. Data are expressed as mean $\pm$ SEM of saliva absolute values. * $p < 0.05$; ** $p < 0.005$ vs. healthy women (Tukey's post hoc test); *** $p < 0.008$ vs. healthy women (one-way ANOVA).

($F_{4,72} = 3.42$, $p < 0.015$), indicating that the time pattern of saliva ghrelin response to TSST significantly differed between the BN individuals and the healthy women. Indeed, while saliva ghrelin reached a peak 25 min after the TSST and then rapidly decreased in the control women, in the BN patients saliva ghrelin concentrations remained high up to the end of the observation period. Moreover, in the BN patients the saliva ghrelin levels 25, 40 and 60 min after the TSST test were significantly higher than in the healthy women (fig. 2). The saliva ghrelin response to TSST evaluated as the area under the curve (AUC) was significantly higher in the BN patients than in the healthy women ($F_{1,18} = 5.44$, $p < 0.04$) (fig. 2).

Correlations

No statistically significant correlations emerged between BITE and TFEQ scores and saliva ghrelin and cortisol AUCs in both the patients and controls. In the BN patients no significant correlations emerged between the weekly frequency of bingeing, the duration of the illness and the AUCs of both saliva ghrelin and cortisol.

Discussion

In symptomatic BN individuals we found normal pre-stress levels of salivary cortisol and normal activation of the HPA axis by a psychosocial stressor. However, although the pre-stress levels of salivary ghrelin did not differ between the BN patients and the healthy women, the ghrelin response to TSST was significantly longer and higher in the BN patients than in the controls. Indeed, in both groups, salivary ghrelin progressively rose after the TSST to reach a peak after 25 min; however, while in the healthy women salivary ghrelin concentrations returned to baseline values 40 min after the stressor, in BN patients they were still significantly high 40 and 60 min after the TSST. To the best of our knowledge, this is the first study concomitantly exploring cortisol and ghrelin reactivity to a psychosocial stressor in symptomatic patients with BN.

Although not all the studies agree, increases of cortisol and ghrelin production have been detected in both animals and humans in response to acute stressors, as is the case in the present study. Indeed, a rise in either gastric ghrelin mRNA or total plasma ghrelin after acute stress was reported in rodents [15, 16]. Similarly, a significant

TSST-induced increase of circulating ghrelin was detected in both normal-weight healthy subjects and obese persons, with or without binge eating, who displayed a concomitant TSST-induced increase in plasma cortisol, but not in those whose plasma cortisol levels did not rise after the stress procedure [26]. Zimmermann et al. [27], on the other hand, did not observe any TSST-induced increase of circulating ghrelin concentrations in healthy male volunteers in spite of their stress-induced increase of circulating cortisol levels.

While it has been largely documented that salivary cortisol is responsive to stress and closely reflects the concentrations of free cortisol in plasma [28], the extent to which salivary ghrelin mirrors circulating concentrations of the hormone is still a matter of debate. In fact, although several studies reported a positive correlation between salivary and serum ghrelin levels in both healthy subjects [29–31] and patients with ischemic heart disease or end-stage renal disease [32, 33], one recent research could not find such a correlation in patients with cancer or obesity [31]. Moreover, Dynesen et al. [34] did not detect significant correlations between meal-induced changes in saliva and serum ghrelin concentrations in both healthy women and patients with BN. Although at present there is no certainty that salivary ghrelin reflects circulating ghrelin, our findings show that salivary ghrelin is responsive to a psychosocial stressor in both healthy women and female patients with BN, with a significantly increased and prolonged response of the hormone in the latter group. Since ghrelin and its two receptor isoforms have been shown to be produced by the human salivary glands and secreted into saliva [35], it could be assumed that the enhanced stress-induced production of salivary ghrelin in our BN patients could be related to the usually observed hypertrophy of their salivary glands. However, this explanation seems unlikely since no significant difference in both basal and meal-induced changes in saliva ghrelin levels has been reported between healthy controls and symptomatic BN patients [34] as well as in the pre-stress levels of salivary ghrelin in our subjects.

If one assumes that stress-induced changes in salivary ghrelin reflect similar changes in the blood, some hypotheses can be put forward to explain the teleological significance of ghrelin increase after stress exposure. It has been recently proposed that ghrelin may mediate the rewarding aspects of eating that are separated from eating-associated body weight homeostasis [36] and, therefore, its rise after stress exposure could be involved in stress-induced feeding where the ingestion of food is

aimed at reducing negative emotions by increasing food-derived feelings of pleasure. Therefore, since in our BN patients the ghrelin secretion in response to TSST was amplified, we could speculate that when BN patients are in conditions of negative mood states, the psychological distress may induce an exaggerated ghrelin secretion which could constitute a strong signal of hunger and/or urge to eat, thereby prompting binge eating. This would reduce the patients' anxiety and/or negative emotions by increasing feelings of pleasure derived by the ingestion of food. Unfortunately, we did not measure the hunger and/or urge to eat sensations in our participants after the stress procedure, and we could not detect any significant correlation between the ghrelin response to TSST and clinical measures of binge eating such as the patients' weekly frequency of bingeing and BITE and TFEQ scores. Moreover, the literature data show that in both healthy controls and obese subjects, with or without binge eating, reporting an increase in their urge to eat after a TSST, no significant correlation was found between the post-stress increase in the urge to eat and changes in plasma ghrelin levels, although the AUC of plasma ghrelin was marginally higher in subjects who reported an increased urge to eat compared to those who did not [26]. Therefore, the role of stress-induced ghrelin secretion in mediating binge-eating behaviour remains to be ascertained.

The present findings of normal pre-stress levels of salivary cortisol and normal stress-induced activation of the HPA axis in symptomatic BN women are consistent with a large body of the literature. They are also consistent with our previous study which showed a normal HPA axis basal activity and a physiological response to TSST in the acute phase of BN [19, 37]. Therefore, the present data provide further evidence of an intact HPA axis activity in symptomatic BN patients, although increased HPA functioning has also been reported in the acute phase of BN [19]. Epel et al. [37] explored the relationship between stress-induced salivary cortisol increase and food ingestion. Their study found that premenopausal women who could be classified as high cortisol reactors to a modified version of the TSST consumed more calories on the stress day compared to low reactors but ate similar amounts on the control day. This suggests that individual differences in salivary cortisol responses to TSST might differently affect stress-induced food intake. The low number of subjects included in this study did not allow us to distinguish between high and low cortisol reactors. In addition, we were unable to analyse if their salivary ghrelin responses could depend on an

appropriate cortisol response. This is in view of the above-reported significant TSST-induced increase of circulating ghrelin in subjects displaying a concomitant TSST-induced increase in plasma cortisol, but not in those whose plasma cortisol levels did not rise after the stress procedure [26].

Some limitations of this study need to be discussed. The first and most relevant of these is that we measured 'total' ghrelin, that is, both acyl and desacyl ghrelin. Acylation of ghrelin is likely essential for its biological activity. We cannot exclude that the TSST could affect acyl and desacyl ghrelin differently. The second limitation is represented by the relatively low number of subjects in each group, which could be responsible for false positive results because of the high inter-individual variability in the hormonal responses to stressors. Therefore, although

the difficulty in collecting larger samples for this type of study is clearly intuitive, the present results should be regarded as preliminary.

In conclusion, compared to healthy women, symptomatic bulimic women displayed normal cortisol but enhanced ghrelin responses when exposed to an acute psychosocial stress. The extent to which the deranged stress-induced ghrelin secretion could contribute to the binge-eating behaviour of BN patients remains to be clarified.

Disclosure Statement

The authors declare that they have no conflicts of interest.

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